EXPERIMENTAL STUDIES ON THE INFLUENCE OF NITROMIN PREMEDICATION ON THE TRANSPLANTATION OF THE YOSHIDA SARCOMA

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by

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I INTRODUCTION

We often experience that during a treatment for tumor with today's anticancer drugs, the growth of malignant tumor is for a while inhibited, but it sooner or later becomes active again and death follows at last. About this phenomenon, the fact of acquired resistibility of tumors against anticancer drugs and selective proliferation of unsusceptible tumor cells against them have been clarified; this is an approach attempted primarily from the side of the tumor cells. The influence of changed internal circumstances by general weakness or cancerous cachexia on tumor growth is still obscure. Such factors as immunological reaction and disturbance of liver function etc. may have some influences on tumor growth.

The author suspected that the side effect of anticancer drugs might decrease the resistibility of organisms against the tumor growth.

According to some reports, mustard group acts carcinogenically in spite of its having destructive action on tumor cells and promotes tumor growth on some occasion.

In this paper the influence of NITROMIN (Nitrogen Mustard N-oxide Hydrochloride) on the "take" of the tumor transplantation was examined experimentally, applying the drug previous to the tumor transplantation.

II MATERIALS

Animals : Wistar-rats from the Inbred Animal Center of Kyoto University, weighing about 100g.

NITROMIN : For intraabdominal injection, 1% solution in dose of 10mg/kg or 20 mg/kg were used.

For subcutaneous injection, 0.1% solution in dose of 1mg/kg or 2mg/kg were used.

NITROMIN, dissolved in saline solution, was kept in an ice-box and used within 24 hours. Its minimal effective doses (M. E. D.) for the YOSHIDA sarcoma in rat were reported to be 1mg/kg per week.

Tumor : YOSHIDA sarcoma. Tumor cells were collected at the time when their proliferation reached maximum in strains of tumor-bearing, untreated rats.

Ⅲ EXPERIMENT Ⅰ

THE INFLUENCE OF VARIOUS DOSES OF NITROMIN ON BODY WEIGHT AND LEUKOCYTE COUNT OF RAT AND ON THE TRANSPLANTED TUMOR

METHOD

About 5 rats in a group were weighed every day and injected with NITROMIN. Some of them were examined for leukocyte count in blood obtained from the tail vein. Some days after the final injection, the YOSHIDA sarcoma was injected intraabdominally and survival days were observed. These experiments were repeated to confirm the observation.

Such groups in which many animals died within 4 days after the transplantation were excluded, because the survival days were too short (average survival days in control rats were 8.9 days) and because in such groups the intraabdominal tumorous changes were too scanty to ascribe the death to the tumor.

RESULTS

Changes in leukocyte count and body weight were shown in figures and tables. Leukocyte counts were measured at certain intervals; to show the daily change in leukocyte count graphically the curve for corrected averages was drawn. For example, in Figure 1, the average leukocyte count on the 5th day was quoted from that of Figure 2 and the dotted line in Figure 2 was drawn by presumption. These corrected curves show that the leukocyte count decreases during the period of successive injections of NITROMIN. In Figure 2, average body weight on the 8th day was given also as corrected. Figures for body weight were drawn only on the basis of the cases whose initial body weights were 100—110g.

1) Subcutaneous injections of 1mg/kg of NITROMIN daily for four successive days (Table 1, Figure 1).

The day next to the 4th injection, the leukocyte count decreased to 46—62% level (average 54%) as shown in Table 2; but on the 2nd day after the last injection it returned to about the normal value.

During the period of injections body weight increased by zero to 10g (average increase 4g) and no decrease was seen in general.

According to a report[1], if rats with the YOSHIDA sarcoma were injected with 1mg/kg of NITROMIN daily more than for two days, the tumor growth was inhibited for 5—7 days thereafter. In the present experiment, however, the rate of lethal take, when transplantation was done two days after the injections, was 5/6 (83%) and average survival days were 9.2 days. In the control group, the rate of lethal take was 177/237 (75%) and average survival days were 8.9 days. Neither tumor growth inhibition nor evident prolongation of survival days were observed in the present experiment and there was no visible anticancer effect of premedication with NITROMIN at this dose level.

Figure 1. CHANGES OF BODY WEIGHT AND LEUKOCYTE COUNT
Subcutaneous Injection of 1mg per Kg Body Weight
of NITROMIN daily for Four Successive Days

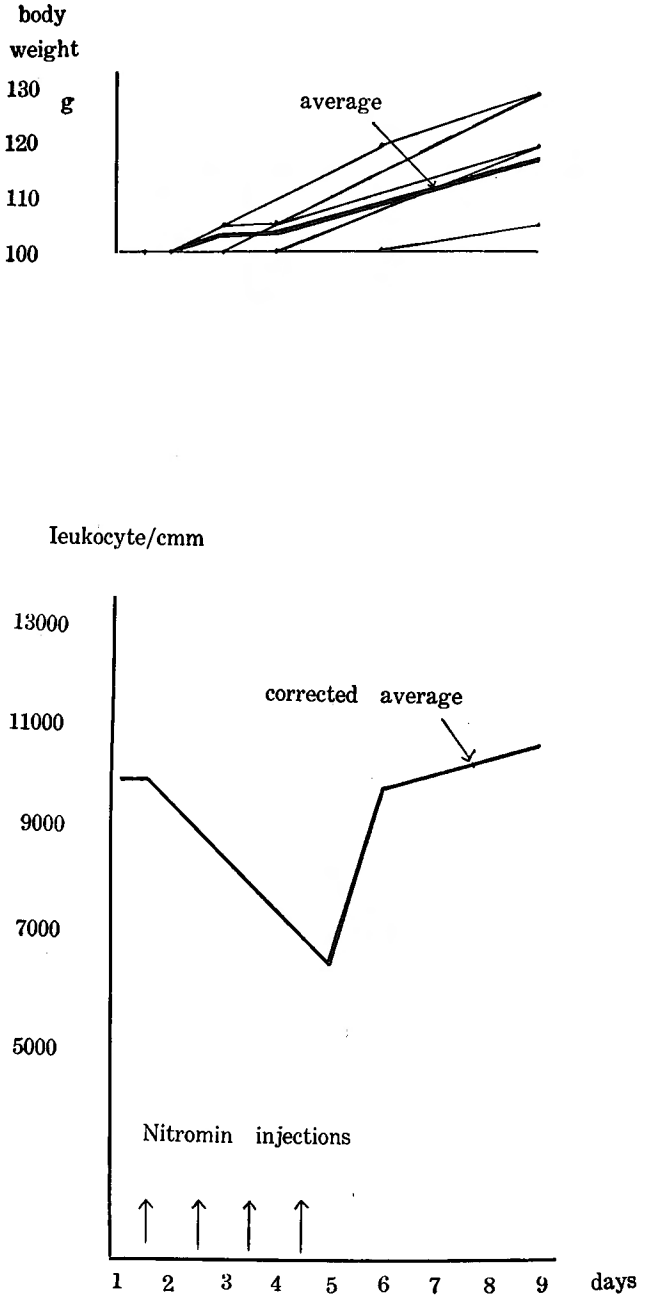


Table 1. CHANGES OF BODY WEIGHT AND LEUKOCYTE COUNT

Subcutaneous Injections of 1 mg per Kg Body Weight of Nitromin daily for Four Successive Days

No.of rat		days 1	2	3	4	6	9	survial days	omental tumor	ascites
1	l.c.	10000				9000	8000 ↓	4	+	+
	b.w.	100	100	100	105	115	130			
2	l.c.	10200				11800	14400	8	+	+
	b.w.	100	100	100	100	100	100			
3	l.c.	10200				9800	9400	5	+	+
	b.w.	100	100	105	110	120	130			
4	l.c.	12200				9800	9200	11	+	+
	b.w.	95	95	95	100	100	115			
5	l.c.	7200				8000	12000	4	+	+
	b.w.	100	100	100	100	100	105			
6	b.w.	120	110	120	120		140	7	+	+
7		100	100	105	105		120	8	+	+
8		110	115	115	110		130	9	+	+
9		100	100	105	105		120	10	+	+
10		100	100	105	105		120	11	+	+
11		100	100	100	100		120	7	+	+

the group transplanted 5 days after injections
average survival days·····7.6 days
lethal take (%) ·····11/11 (100%)

1					↓		7	+	+
2	90			90			8	+	+
3	⋮			⋮			10	−	−
4	100			100			10	+	+
5							10	+	+
6							11	+	+

the group transplanted 2 days after injections
average survival days·····9.2 days
lethal take (%) ·····5/6 (83%)

l. c. : leukocyte count /cmm
b.w. : body weight (g)
↓ : YOSHIDA sarcoma transplantation

When transplantation was done 5 days after the last injection, the rate of lethal take was 11/11 (100%) and survival days were shortened to 7.6 days in average. In this group, 2 rats which died within 4 days after transplantation showed already evident omental tumor.

It is quite interesting that the rate of lethal take became higher and survival days were shortened when transplantation was done on 5th day after the last injection.

The average leukocyte count on the 5th day after the last injection was, as shown in Figure 1, higher than that during the initial stage. If a decrease in leukocyte count should indicate the activity of NITROMIN, it is thought that this activity has disappeared by the 5th day after the last injection, so that the rate of lethal take is expected to be at least equal to that of the control group; contrary to this assumption, the fact was as stated above. The author tried other experiments taking notice of this phenomenon.

2) Subcutaneous injections of 1mg/kg of NITROMIN daily for seven successive days

Figure 2. CHANGES OF BODY WEIGHT AND LEUKOCYTE COUNT
Subcutaneous Injections of 1mg per kg Body Weight of
NITOROMIN daily for Seven Successive Days

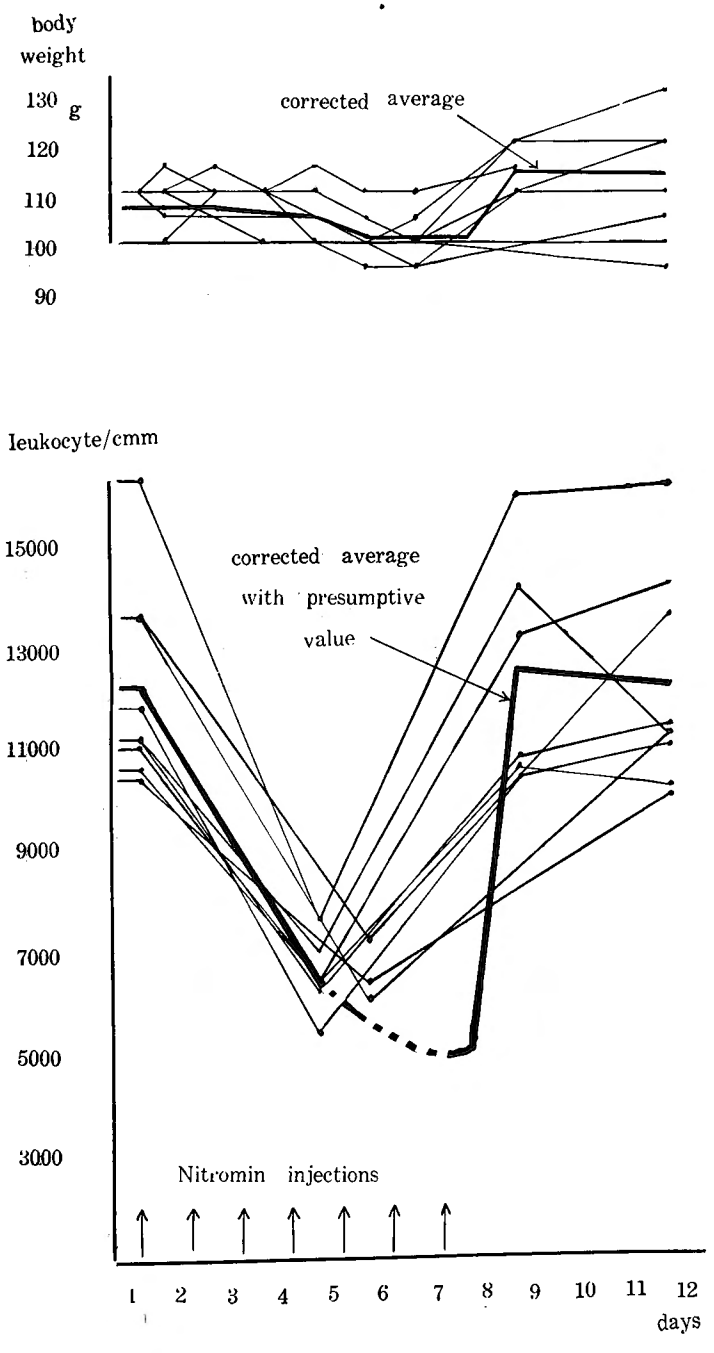


Table 2. Subcutaneous Injections of 1 mg per Kg Body Weight of Nitromin daily for Seven Successive Days

No.of rat		days 1	2	3	4	5	6	7	……9……12……		survival days	omental tumor	ascites
1	l. c.	11200				6200			10800	11400	10	+	+
	b.w.	110	110	110	110	100	95	95	110	110↓			
2	l. c.	11200				7000			14200	11200	12	+	+
	b.w.	110	110	110	110	105	100	105	120	120			
3	l. c.	16300				7600			16000	16200	10	+	+
	b.w.	110	105	105	100	100	100	100	110	120			
4	l. c.	11800				5400			10400	11000	12	+	+
	b.w.	110	115	110	110	110	105	100	120	130			
5	l. c.	11000				6400			13200	14200	alive		
	b.w.	110	110	105	105	105	100	100	110	120			
6	l. c.	10600				6400			10600	10200	10	+	+
	b.w.	115	115	120	120	125	120	120	130	140			
7	l. c.	13600					6000			11200	9	+	+
	b.w.	100	100	100	100	100	100	100		100			
8	l. c.	10400					6400			10000	7	+	+
	b.w.	100	100	100	100	100	100	95		105			
9	l. c.	13600					7200			13600	5	+	+
	b.w.	100	100	110	110	105	100	100		95			

the group transplanted 5 days after injections
average survival days ……9.4 days
lethal take (%) ……8/9 (89%)

1		120	120	120	120	125	125	120	130	alive		
2		120	120	120	120	125	125	125	130↓	15	+	+
3		120	120	120	120	120	120	120	120	8	+	+
4		120	115	120	115	115	115	115	120	8	+	+
5		110	110	115	110	115	110	110	115	alive		
6		90						90		9	+	+
7		⋮						⋮		10	+	+
8		100						100		10	+	+
9										10	+	+
10										11	+	+
11										11	+	+

the group transplanted 2 days after injections
average survival days---10.2 days
lethal take (%) -------9/11 (82%)

(Table 2, Figure 2).

In 14 cases in which tumor was transplanted 2 days or 5 days after injections differences of body weight between the pre- and postinjection period were from -15g to 5g (average decrease 3.6g). The average leukocyte count decreased to less than 53% level, (on the 8th day leukocyte counts were not counted. Leukocyte count decreased on the 5th day and the 6th day of successive injections and the degree of decrease was greater when larger doses of NITROMIN were used. From these facts it was thought that on the 8th day leukocyte count decreased more than on the 5th day or the 6th day as shown by hypothetical curve in Figure 2) ; on the 2nd day after the final injection leukocyte count returned to normal value and the body weight increased a little.

Rats transplanted 2 days after the last injection survived in ratio of 2/11 18% and

Table 3 Subcutaneous Injections of 2 mg per Kg Body Weight of Nitromin daily for Four Successive Days

No.of rat	days 1	2	3	4	5	6----9	survival days	omental tumor	ascites
1 l. c.	15600				6800	16200 ↓	5	+	+
b.w.	90	90	90	95	90	95 ↓			
2 l. c.	11600				6000	10000	6	+	+
b.w.	85	85	80	85	75	85			
3 l. c.	13600				12000	16200	6	+	+
b.w.	90	95	95	100	95	100			
4	90	95	95	95		100	4	−	−
5	90	85	85	85		95	6	+	+
6	90	100	100	100		100	7	+	+
7	90	90	90	90		90	8	+	+
8	90	90	90	90		95	9	+	+

the group transplanted 5 days after injections
average survival days ---7 days
lethal take (%) --------7/8 (88%)

1	90	85	85	90	100 ↓		6	+	+
2	90	90	90	95	100		alive		
3	85	85	85	90	90		9	+	+
4	90	85	90	90	95		9	+	+
5	90	85	90	90	90		8	+	+
6	90	90	90	100	110		alive		
7	100			100			6	−	−
8	⋮			⋮			6	+	+
9	⋮			⋮			8	+	+
10	120			120			8	+	+
11							15	+	±

the group transplanted next day after injections
average survival days ---8.6 days
lethal take (%) --------8/11 (73%)

alive : more than a month

died of tumor in ratio of 9/11 (82%) ; average survival days were 10.2 days.

Rats transplanted 5 days after the last injection survived in ratio of 1/9 (11%) and died of tumor in ratio of 8/9 (89%) ; average survival days were 9.4 days. Comparing these data with that of the control group (the rate of lethal take, 75% ; average survival days, 8,9 days), no clear anticancer effect of injected NITROMIN was seen even in the group transplanted 2 days after the final injection.

3) Subcutaneous injections of 2mg/kg of NITROMIN daily for four successive days (Table 3)

Differences of body weight between the pre- and postinjection period were from -5g to 10g (average increase 3.2g). In this group transplantation was done on the next day after the 4th injection when leukocyte count still decreased. The survival rate was 2/11 (18%) ; the rate of lethal take was 8/11 (73%) ; average survival days were 8.6 days, so that the transplantation rate during this leukopenic stage was almost the same as in the control group, and the survival days were not prolonged. Then transplantation

Table 4. Intraabdominal Injections of 10 mg per Kg Body Weight of Nitromin daily for Four Successive Days

No.of rat	days 1	2	3	4	6	9	11	survival days	omental tumor	ascites
1 l. c.	10000				5400	11000	10800 ↓	7	+	+
b.w.	100	100	95	90		100	100 ↓			
2 l. c.	12000				3000	15600	14400	8	+	+
b.w.	90	90	90	80		100	100			
3 l. c.	11600				3200	6400	11000	6	death due to accident	
b.w.	90	90	85	80		75	70			
4 l. c.	9400				6200	9800	10800	alive		
b.w.	85	85	85	80		90	90			
5 l. c.	11000				3800	9400	14400	8	+	+
b.w.	100	100	90	90		90	90			
6	105	100	90	85	80	90	90	alive		
7	110	100	95	90	80	80	90	13	+	+
8	110	105	100	95	90	115	115	9	+	+
9	110	100	95	90	75	100	100	12	+	+
10	100	95	90	80	70	85	90	10	+	+
11	110	105	100	90	80	100	100	13	−	−
12	105	100	95	90	80	95	90	11	+	+

the group transplanted 7 days after injections
average survival days……9.8 days
lethal take (%) ……8/11 (73%)

1 l. c.	12400				7600	7200 ↓	5	−	−
b.w.	100		90	85	80	90 ↓			
2 l. c.	14800				6400	12000	9	+	+
b.w.	100		100	95	85	90			
3	90			80			4	+	±
4	⋮			⋮			4	+	±
5	110			100			4	+	±
6							8	+	+
7							11	+	+
8							15	+	+
9							16	+	+

the group transplanted 5 days after injections
average survival days……8.9 days
lethal take (%) ……8/9 (89%)

was done on the 5th day after the last injection when the leukocyte count had already recovered. The rate of lethal take was 7/8 (88%) and average survival days were shortened to 7 days.

4) Intraabdominal injections of 10mg/kg of NITROMIN daily for four successive days (Table 4, 5 Figure 3)

As the NITROMIN premedication in dose of 1mg/kg daily for 7 days or 2mg/kg daily for 4 days did not show any anticancer effect, the author tried doses of 10mg/kg daily for 4 days intraabdominally. The maximum tolerable dose (M. T. D.) of NITROMIN for rats is reported to be 40mg/kg per week.

Differences of body weight between the pre- and postinjection period were from −25g to −5g (average decrease 18g) ; all cases decreased in body weight and on the

Table 5. Intraabdominal Injections of 10 mg per Kg Body Weight of Nitromin daily for Four Successive Days

No.of rat	days 1	2	3	4	5	6	survival days	omental tumor	ascites
1 b.w.	110	105	100	95		80 ↓	14	+	+
2	100	90	90	80		70	12	−	−
3	85	85	80	80		75	18	−	−
4	90	85	85	75		70	2	−	−
5	90	60	95	75		60	alive		
6	130	130	120	110		120	12	+	+
7	100	100	100	95		100	12	+	+
8	110	110	100	90		100	alive		
9	120	110	105	95		105	alive		
10	105	105	100	90		90	alive		
11	110	100	100	90		90	alive		
12	120					90	4	−	−
13	120					⋮	4	−	−
14	120					⋮	5	−	−
15	120					⋮	7	+	+
16	120					⋮	10	+	+
17	120					110	14	−	−

the group transplanted 2 days after injections
average survival days·····11 days
lethal take (%) ·····5/17 (29%)

2nd day after the last injection the body weight still decreased and the leukocyte count decreased to 44% level on average; but none died of acute poisoning at all.

In the group transplanted 2 days after the last injection, the survival rate was 5/17 (29%) ; the rate of lethal take was 5/17 (29%) and the rest i. e. 7/17 (41%) died without intraabdominal findings. In this group, cases of typical death from tumor were fewer than in the control group and the average survival days were prolonged to 11 days.

In the group transplanted 5 days after the last injection, the rate of lethal take was 8/9 (89%) ; average survival days were 8.9 days.

In the group transplanted 7 days after the last injection, the rate of lethal take was 8/11 (73%) ; average survival days were 9.8 days.

From the entire experiments, the following statement can be made. In the group transplanted 2 days after the last injection, tumorous findings were inhibited and some cured, while in the group transplanted 5 days after the last injection the lethal take occurred in higher rates ; in the group transplanted 7 days after the last injection the situation was almost the same as in the control group.

Changes of leukocyte count and body weight showed that toxic actions of NITROMIN were still remaining on the 2nd day after the last injection and both leukocyte count and body weight decreased ; on the 5th day after the last injection, however, these values returned to about normal and on the 7th day they increased slightly more.

5) Intraabdominal injections of 20mg/kg of NITOROMIN daily for four successive days (Table 6, 7 Figure 4).

Figure 3. CHANGES OF BODY WEIGHT AND LEUKOCYTE COUNT

Intraabdominal Injections of 10mg per kg Body Weight of NITROMIN daily for Four Successive Days

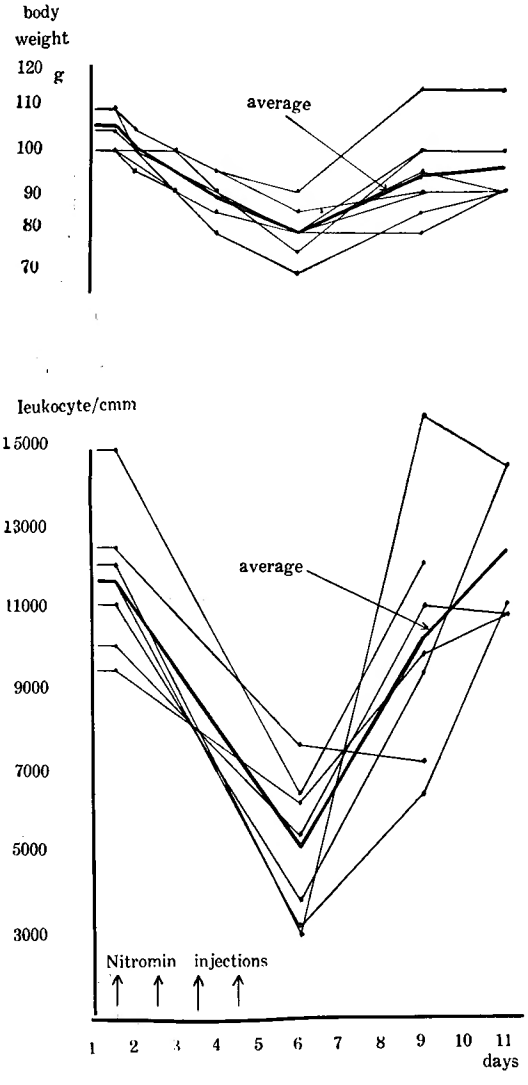
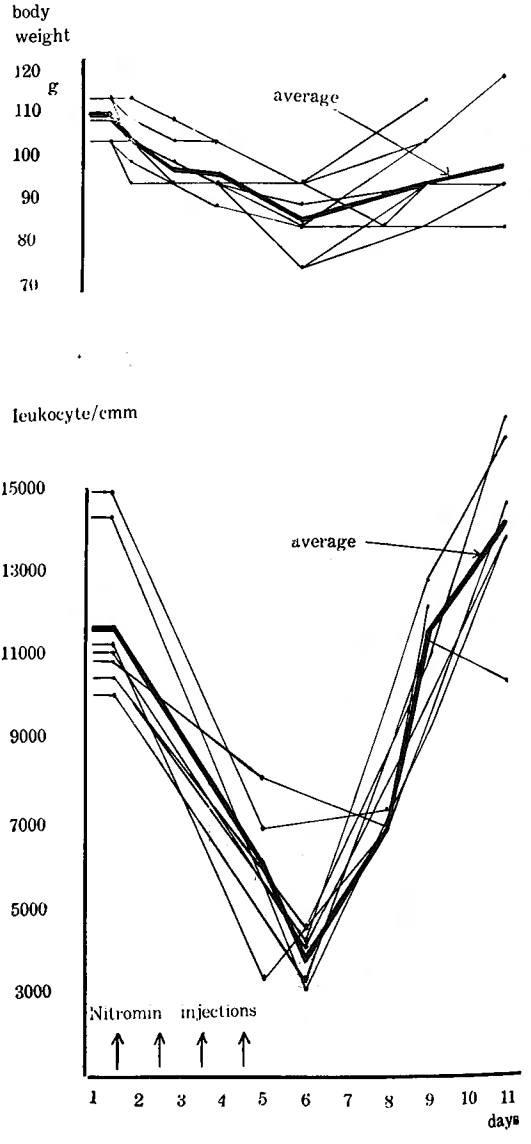


Figure 4. CHANGES OF BODY WEIGHT AND LEUKOCYTE COUNT

Intraabdominal Injections of 20mg per kg Body Weighf of NITROMIN daily for Four Successive Days



LD_{50} of NITROMIN for rats is reported to be 80mg/kg per week, so these doses were applied being divided into 4 equal injections.

Differences of body weight between the pre- and postinjection period were from −20g to zero (average decrease 14g) ; all cases decreased in body weight. On the 4th day after the last injection, body weight and leukocyte count were not recovered as yet. Leukocyte count recovered to normal value on the 5th day and it exceeded the normal a

Table 6. Intraabdominal Injections of 20 mg per Kg Body Weight of Nitromin daily for Four Successive Days

No.of rat	days 1	2	3	4	5	6……8	9……11	survival days	omental tumor	ascites
1 l. c.	10000					3200	11200 10200 ↓	4	+	+
b.w.	105	100	95	90		90	100 115 ↓			
2 l. c.	10400					4400	10600 16400	9	+	+
b.w.	95	90	80	80		75	85 90			
3 l. c.	11000					4000	9800 13600	alive		
b.w.	90	85	80	75		70	80 90			
4 l. c.	14200					3000	9000 13600	4	−	−
b.w.	95	90	80	80		65	70 70			
5 l. c.	10400					4100	12600 16000	11	+	+
b.w.	90	85	80	75		70	70 85			
6 l. c.	11200									
b.w.	90	85	75	70		†		died of poisoning		
7 b.w.	105	100	90	90		85	90 90	11	+	+
8	110	100	95	90		†		died of poisoning		
9	110	100	90	90		80	80 90	10	+	+
10	100	95	90	80		75	60 †	died of poisoning		
11	100	100	90	85		70	60 50	1 died of accident		
12	100	95	90	90		80	80 80	12	+	+
1 l. c.	11200				3200		6800 12000 ↓	11	+	+
b.w.	110	110	105	100	95		80 80 ↓			
2 l. c.	10800				8000		6800 14400	12	+	+
b.w.	120	120	110	110	100		100 95			
3 l. c.	14800				6800		7200			
b.w.	110	110	100	100	90		80 †	died of poisoning		
4 b.w.	100	100	90	85			80	3	−	−
5	120	120	115	110			100	8	+	+
6	110	100	90	90		70	80	10	+	+
7	100	100	90	90		70	90	10	+	+
8	100	90	90	85		80	100	10	+	+
9	110	105	100	100		90	110	12	+	+
10	95	85	80	75		65	60	3	+	+
11	100	90	85	80		70	†	died of poisoning		

little on the 7th day.

Five cases out of 34 died of acute poisoning. In the group transplanted 2 days after the last injection, the survival rate was 6/10 (60%) ; the rate of lethal take was 4/10 (40%), so the cure effect of NITROMIN was recognizable in this group.

In the group transplanted 5 days after the last injection, however, the rate of lethal take was 8/9 (89%), and average survival days were 9.5 days. In the group transplanted 7 days after the last injection. the rate of lethal take was 6/8 (75%) and this was almost the some as in the control group. Summary of these facts is as follows.

On the 2nd day after the last injection the leukocyte count decreased evidently and at that stage 60% of the rats did not die of the YOSHIDA sarcoma even though they were transplanted with the tumor intraabdominally. On the other hand, on the 5th day after the last injection leukocyte count recovered and the rate of lethal take became higher than that of the control group but the survival days were from 5 days to 12 days. i. e., not always shortened. This difference of survival days appears to be due to varying

Table 7. Intraabdominal Injections of 20 mg per Kg Body Weight of Nitromin daily for Four Successive Days

No. of rat	days 1	2	3	4	5	6	survival days	omental tumor	ascites
1 b.w.	120	110	110	100		105↓	alive		
2	105	100	100	90		85	9	+	+
3	95	95	90	90		85	5	died of accident (excluded)	
4	90	95	95	90		90	alive		
5	90	90	90	80		80	9	+	+
6	120	110	105	100		90	10	−	+
7	100	100	90	85		90	alive		
8	110	115	110	105		105	alive		
9	100	95	90	80		80	alive		
10	105	105	100	95		95	11	+	+
11	110	115	110	100		100	alive		

the group transplanted 2 days after injections
average survival days……9.8 days
lethal take (%) ……4/10 (40%)
the group transplanted 5 days after injections
average survival days……9.5 days
lethal take (%) ……8/9 (89%)
the group transplanted 7 days after injections
average survival days……9.5 days
lethal take (%) ……6/8 (75%)

sensitivity of each rat to NITROMIN. There was something about this group which distinguished this group from others.

On the 7th day after the last injection, effect of NITROMIN seemed to have disappeared, because the rate of lethal take was almost the same as that of the control group.

Ⅳ EXPERIMENT Ⅱ

EFFECT OF NITROMIN ON NEGATIVE TAKE RATS (Table 8, 9)

In a group with subcutaneous injections of 1mg/kg of NITROMIN daily for 4 successive days, the rate of lethal take with typical tumorous findings was 100% when transplanted 5 days after injections. When the tumor was transplanted 5 days after successive injection of NITROMIN in various dose, the total transplantation rate was 42/46 (91%) and it was significantly higher than that of control group, 177/237 (75%). ($\frac{d}{\sigma_d}=3.15>2$, $\chi^2=6.08>4$, $p=0.0143$). From these facts, a tumor growth promoting effect of the NITROMIN premedication was suspected. Or else, changed internal circumstances of host by the NITROMIN premedication might have favored the tumor growth. To examine this point, next experiment was undertaken.

Subcutaneous injections of 1mg/kg of NITROMIN daily for 4 successive days were done in 7 negative take rats and 5 days after the last injection the tumor was transplanted but no positive take occurred at all. Other 2 negative take rats were treated with intraabdominal injections of 20mg/kg of NITROMIN daily for 4 successive days and transplanted

Table 8. NEGATIVE TAKE GROUP

Subcutaneous Injections of 1mg per Kg Body Weight of Nitromin daily for Four Successive Days

No. of rat	days 1	2	3	4	 9	take
1 b. w.	235	235	240	240	240 ↓	—
2	180	180	175	170	175	—
3	170	165	165	165	170	—
4	160	155	160	150	150	—
5	190	190	190	195	200	—
6	180	180	170	170	170	—
7	180	180	180	170	175	—

All cases survived more than 4 weeks.

Intraabdominl Injections of 20mg per Kg Body Weight of Nitromin daily for Four Successive Days

No. of rat	days 1	2	3	4	 11	take
1 b. w.	160	150	140	130	↓	—
2	170	160	140	135		—

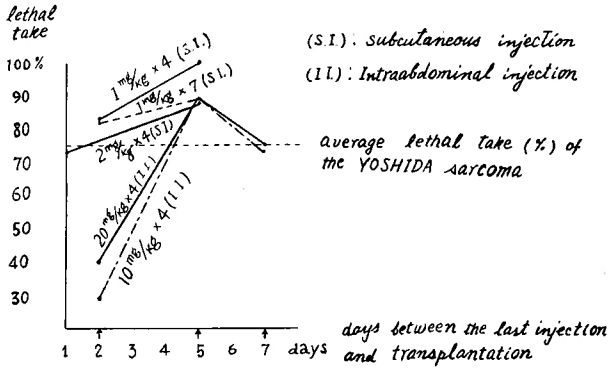
All cases survived more than 4 weeks.

Table 9. The group transplanted 5 days after successive injection of NITROMIN in various dose.

amount (mg/kg)	times	method	rate of lethal take	per cent
1	4	subcutaneously	11/11	100%
1	7	subcutaneously	8/9	89%
2	4	subcutaneously	7/8	88%
10	4	intraabdominally	8/9	89%
20	4	intraabdominally	8/9	89%
		total	42/46	91%

Rate of lethal take of the Yoshida sarcoma in each month.

month	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Total
rate	10/13	12/16	26/34	25/34	25/34	24/32	33/45	22/29	177/237
%	77	75	76	74	74	75	73	76	75
average survival days	8.3	8.8	8.0	9.8	9.0	9.3	8.3	9.0	8.9



on the 7th day after the last injection but all cases survived. It follows that rats with a congenital resistibility against the tumor could not be changed into susceptible ones by these premedications. The fact that the rate of lethal take was 100% in a group above mentioned seems to be due to the absence of unsusceptible rat in that group by chance.

V EXPERIMENT III

DEATH FROM ACUTE NITROMIN POISONING (Table 10).

It is said that in non-inbred rats the survival days after the transplantation of the YOSHIDA Sarcoma are from 7day to 12 days; in 177 cases of Wistar-rats in the present experiment the average survival days were 8.9 days. But during the experiment some died in 3 to 6 days and, while some of them had intraabdominal findings, others had not. In the following experiment the survival days before death from NITROMIN poisoning was examined.

Table 10. Death from Acute Nitromin Poisoning.

No. of rat	leukocyte count (per cmm)	body weight (g)	amount of Nitromin (mg)	survival days
1	12200	100	10	alive
2	14400	90	9	6
3	15300	85	8.5	4
4	15200	90	9	3
5	10400	100	10	2

Five rats weighing from 85g to 100g were injected with 100mg/kg of NITROMIN intraabdominally (minimal lethal dose for rats is reported to be 130mg/kg per week). Four out of five (80%) died between 2 and 6 days after injection, and only one survived. Therefore the NITROMIN tolerance of rats was quite different in each individual and death from poisoning appeared within about a week. So some of the cases which died within a week in the experiment above mentioned seemed to be due partly to tumor and partly to poisoning; especially the cases which died without tumorous findings seem to have died of poisoning.

VI DISCUSSION

The YOSHIDA sarcoma is so sensitive to NITROMIN that its growth is, according to a report, evidently inhibited when 1mg/kg per day of NITROMIN is injected more than for 2 days successively; on the other hand, it is known that the premedication of NITROMIN before hepatoma induction by azo-dye in rats accelerates the induction of the tumor[2] and mustard group induces the lung cancer in mice at a high production rate[3].

The author noted that the human tumor proliferated in spite of the NITROMIN treatment and he was interested in the action of NITROMIN not only from the viewpoint of its action on tumor cells but also on host. In this paper, examinations were carried out to know which of these actions was stronger when tumor was transplanted after successive injections of NITROMIN, and whether leukopenia and general weakness following NITROMIN injection could cause the decreased resistibility of the host against tumor growth or not. Only some cases wich were transplanted 2 days after injections of 10mg/kg or 20mg/kg of NITROMIN daily for 4 days were cured, but no effect was found when doses of 4-8 times of the minimal effective dose (1mg/kg daily) in total were injected before the transplantation. The rate of lethal take was rather high during the convalescence in terms of the leukocyte count. During convalescence not only NITROMIN had possibly been detoxified and lost its anticancer effect in the host but also the host seemed to have become rather vulnerable to tumor growth.

According to reports on histological changes due to NTROMIIN[6][7] given in dose of 1mg/kg - 10mg/kg daily for 7 days, changes in the liver, testes and leukocyte count of rats were transitory and these recovered soon. In the experiment, furthermore, it was observed that the steeper the leukocyte count decrease after injections of NITROMIN, the greater its increase reboundingly during convalescence.

In treatments with anticancer drugs, medication is often compelled to be stopped because of unfavorable side effects of drugs, such as leukopenia and general weakness. In this stage, it seems, the once hindered growth of tumor cells could become re-activated. On the other hand, tumor cells had already acquired resistibility against anticancer drugs and these matters can act together to lead the host to death.

Negative take rats, though they were treated with the same drug, could not be changed into susceptible ones. This suggests that heredo-immunological factors predominate over the effect of the NITROMIN premedication.

VII CONCLUSION

Influence of the NITROMIN premedication on transplantability of the YOSHIDA sarcoma was examined and the following results were obtained.

1) Even when doses of 4-8 times of the minimal effective dose of NITROMIN were injected subcutaneously during a week before transplantation, the growth of the YOSHIDA sarcoma was not inhibited when it was transplanted 2 days or 5 days after the last injection.

2) NITROMIN injected daily into the abdominal cavity in doses of 10mg/kg or 20

mg/kg daily for 4 successive days showed some inhibitory effect on the YOSHIDA sarcoma growth which was transplanted 2 days after the last injection, but no inhibition was seen when sarcoma was transplanted more than 5 days after the last injection.

3) When the YOSHIDA sarcoma was transplanted 5 days after the last injection in various doses, the total transplantation rate was 42/46 (91%) and it was significantly higher than that of the control group, 177/237 (75%).

4) Negative take rats could not be changed into susceptible ones by these premedications.

5) NITROMIN tolerance in rats was quite different in each individual and death from poisoning occurred in a week. Therefore, in the cases of short survival days after the NITROMIN premedication and tumor transplantation, death could be both due to poisoning and due to tumor.

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和文抄録

ナイトロミン前処置の吉田肉腫移植に及ぼす影響についての実験的研究

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ナイトロミンの最小有効量から LD_{50} 迄の一定量を各群のラッテに毎日連続，分割に注射しラッテの体重，白血球数の変動を追求した．次で白血球数減少期，回復中期，回復終了期の3群に分け，各群に吉田肉腫を腹腔内に移植して，ナイトロミン注射後のこれら各期が呈する腫瘍に対する影響を観察し次の結果を得た．

1) ナイトロミンの吉田肉腫に対する最小有効量の4～8倍総量を分割連続皮下注射した場合，注射終了第2日以後の移植吉田肉腫の増殖は抑制且つ阻止されないが，第5日の移植では腫瘍による死亡率が対照よりも高かつた．

2) 最大耐量以上を分割連続腹腔内注射2日後に移植した場合は，腫瘍増殖に対する抑制且つ阻止効果が認められるが，白血球数が回復する第5日以後の移植の場合には，認められなかつた．そして第5日に移植した場合は腫瘍による死亡率が高くなつた．

3) 全例に於て注射5日後の移植では，腫瘍による死亡率が高く，之は対照群に比べて有意の差がある．即ちこの時期には腫瘍の増殖が促進される状況にある事が考えられる．而も之は抗癌剤治療中の休薬期であつて白血球数の回復期が腫瘍増殖の好機になる事を思わせるものである．

4) 移植陰性で生存したラッテに，同様の前処置を行なつて再移植したが，移植陽性には出来なかつた．即ち先天的抗腫瘍性の方がこれらの前処置よりも優位に移植の可否を支配していると思われる．